HERITABLE DISORDERS OF CONNECTIVE TISSUE

II. The Biology of Normal Connective Tissue*

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CONNECTIVE tissues, the supporting structures of the body, include cartilage, ligaments, tendons, fascia, joint capsules, the subepidermal portions (corium) of the skin, important elements of the heart valves and aorta and smaller blood vessels, and finally bone. In general, connective tissue consists of cellular and fibrous constituents embedded in the so-called ground substance. Reference is made to textbooks of histology (e.g., Fig. 45 of ref. 21) for graphic presentation of the structural interrelationships of these elements.

THE CELLULAR ELEMENTS

Virtually all the other endogenous elements of connective tissue are elaborated by the fibroblast, the main cellular constituent of connective tissue, or by its congeners, such as the osteoblast and the chondroblast. Other cellular elements include the mast cell and wandering cells such as macrophages. (The mast cell, which has long been implicated in the formation of heparin, has been thought to be concerned also in the formation of hyaluronic acid.[21]) Very recently some information has accumulated about the enzymatic processes involved in the formation of acid mucopolysaccharides.[20] In general, however, details of the biochemical processes by which the fibroblast manufactures the other elements of connective tissue are obscure.

THE FIBROUS CONSTITUENTS

The fibrous constituents of connective tissue comprise two main groups: collagenous and elastic. The reticulin fiber would be classified by some as a separate category (see below) but most consider it a member of the collagen group although its precise relationship to the classical collagen fiber is moot.

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Collagen must be defined in terms of its properties. It is a fibrous protein occurring in wide, straight, unbranching white bundles which possess high tensile strength and low elasticity. It has characteristic 640 Å periodicity by small angle x-ray diffraction and by electron microscopy. It contains two unique amino acids, hydroxyproline and hydroxylysine. The former is in relatively large amounts and is used in quantitative determination of collagen.[12,18] It contains large amounts of glycine. Aromatic and sulfur amino acids are present only in low concentration.

The biologic importance of collagen in the individual organism and in the phylogenetic series is tremendous. Collagen represents approximately 30 per cent of the total protein of the human body. In the vertebrate animal it is what cellulose is to plants. As the matrix of bone[39b,33] it was referred to as "ossein" in the older literature. Even the vitreous humor of the eye contains fibers with the properties of collagen, the so-called "vitrosin" of Gross.[26] That it is not limited to vertebrates is indicated (as merely one example) by the fact that collagen is the skeleton of the invertebrate sponge familiar in household and other usage.

The economic importance of collagen dates back to prehistory when the manufacture of leather[8] and glue was first undertaken. Isinglass, now almost completely replaced by synthetic substitutes, was also collagenous in origin. It was in leather manufacture that one property of collagen, thermal shrinkage (at 60° to 65° C.) was discovered and used in judging adequacy of tannage.

The importance of collagen in pathology will be evident to a medical audience since the concept of "collagen vascular diseases" has seemingly gained such wide acceptance. Further medical implications appear to be represented by certain of the hereditary disorders of connective tissue, under discussion here.

These implications—biologic, economic, and medical—have been responsible for very extensive investigations of the nature of collagen[1,3,7,9,11] by scientists of diverse interests and perspectives. The result has been, in the past at least, a situation like the elephant which was examined by the six blind men. Recently several excellent symposia have effected a synthesis of the diverse bits of information.

Neither the ultrastructure of collagen nor the mode of its formation,[11m, 40] is established in full detail or beyond debate. A concept which perhaps is most consistent with the information available is as follows: The building blocks of collagen, the tropocollagen unit in the terminology of Gross, Highberger, and Schmitt,[41] is elaborated by the cell, possibly intracellularly, and is extruded into the extracellular environment where ionic and other conditions favor its orderly linear aggregation into the collagen fibril. The tropocollagen unit is thought to have a length (and periodicity) of about 2,000 Å, and the 640 Å periodicity of the finished product is conceived by Schmitt and collaborators as the result of a staggering of the building blocks in their lining-up side to side. The discovery that it is possible to solubilize collagen and then reconstitute it from solution was responsible for much of this concept. Physicochemical studies indicate that the tropocollagen unit is a thin, rigid rod with a molecular weight of 300,000 to 310,000 by osmolarity and by light scattering, and with dimensions

of about 14 Å by 2,000 Å.[25] Although the larger collagen aggregates are very little extensible and in no way approach elastic fibers in this respect, electron optical observations of collagen fibrils[4] indicate that considerable extensibility of these smaller units is possible. Abnormality in the fibrillar organization may be important in the genesis of the abnormal extensibility of tendons, joint capsules, and ligaments, in certain of the heredity disorders of connective tissue.

The carbohydrate content of collagen is low. What is present may be derived from ground substances playing the role of interfibrillary cement. In pure collagen, hydroxyproline is present as about 14 Gm. per 100 Gm. protein (8.6 Gm. per 100 Gm. amino nitrogen) and glycine in roughly twice as great a proportion. The polar side groups of collagen appear to be located at the areas of the bands displayed by x-ray diffraction and by electron microscopy. These are the reactive areas where stains and tanning agents operate.

The relative metabolic inertia of nonsoluble collagen is a very striking feature.[3] The turn-over rate is slightly higher in bone than in tendon, and in younger animals, but much lower than in proteins of cell and plasma. Soluble or extractable collagen,[6a] which according to the trinitarian breakdown of connective tissue elements followed here must be considered part of the ground substance, has a considerably higher rate of turnover.[5] No collagenase comparable to that produced by *Clostridia* has been demonstrated in man with the exception of the extraction by Schmitt and Sizer[27] of material with the properties of collagenase from the cells around embedded collagenous sutures.

Reticulin fibers have most of the same properties as collagen fibers, most important the 640 Å periodicity. The main difference is their small diameters, stainability by silver, and a relatively high concentration of associated polysaccharide. Reticulin fibers predominate in the embryo, and in the adult animal are relatively abundant in parenchymatous organs, in lymph nodes and spleen, around muscle bundles and fat globules, and in association with amorphous material in epithelial basement membranes. The differences from collagen fibers may be a matter of fiber size or diameter of the individual fibrils in a bundle. Given a collagen bundle and a reticulin bundle of the same over-all diameter, the reticulin bundle may stain with silver because it has a great many more component fibrils of small diameter and correspondingly greater total fibril surface area. Staining with silver is a surface phenomenon as demonstrated by Gross. Whether reticulin is precollagen or immature collagen (not to be confused with procollagen or soluble collagen) is perhaps not too important a consideration. The concept of Gross and others that it is not a progenitor of collagen is based on the fact that it is present in the adult organism seemingly without ever being transformed into collagen and that electron microscopic evidence for aggregation of reticulin fibers (of small diameter) into collagen fibers is lacking. Reticulin fibers are present in the adult organism, in the corium, for example, and are possibly present in as great absolute amounts as at any earlier stage in ontogeny. In the adult, however, the reticulin fibers are "swamped" by the preponderance of collagen fibers.

Elastic fibers[28] are large branching refractile structures, light yellow or brown depending on their age; with a high degree of extensibility; indestructi-

bility in relation to heat, drastic pH changes, and enzymes; a low content of polar amino acids; and finally, certain distinctive tinctorial characteristics. The nature of their staining by orcein and related dyes is not at all well understood; the staining bears no relationship to pH and there is no other evidence that salt linkages are involved.[29]

Whereas collagen is present in areas where a pliant but relatively nonextensible building material is necessary (ligaments, tendons, fascia, joint capsules), elastic fibers serve important functions in areas such as the media of the aorta (where they are responsible for the compression chamber, reservoir, or *Windkessel* hemodynamic function of that structure), in the elastic cartilage of the ear, and in ligaments with large elastic components such as those of the foot and the ligamenta flava of the spine.

Chemically, elastic fibers have approximately the same concentration of nonpolar groups, such as glycine, as does collagen. However, there are virtually no polar amino acids such as hydroxyproline, glutamic, and arginine. The relative absence of reactive amino acid side-chains is probably responsible at least in part for the unique properties of elastin.

Much less is known about the ultrastructure of the elastic fiber than of the collagen fiber. Gross has found that the double helical structures earlier described by him[36] in electron photomicrographs of material derived from aorta were in fact not elastic fiber elements but trypsinogen[32] in the enzyme preparation used in removing nonelastic elements. One view[16] of the chemical constitution is that a pro-elastin core is embedded in a matrix of combined pro-elastin and elastomucin. There is produced in the pancreatic islet tissue an elastase[15,28] for which there is an inhibitor produced elsewhere in pancreas. Elastic fibers are moderately susceptible to digestion by trypsin, resistant to digestion by pepsin; this is the converse of the situation with collagen.

THE GROUND SUBSTANCE

The ground substance is the extracellular, extrafibrillar, amorphous matrix of connective tissue. It has components derived from the fibroblast such as acid mucoprotein, acid mucopolysaccharide, and dispersed (soluble or pro-) collagen, and components not elaborated locally such as water, ions, small organic molecules such as glucose, cell metabolites, plasma proteins, and others. It is important not to equate ground substance to acid mucoproteins and acid mucopolysaccharides, as has become a frequent practice, since on a quantitative basis, and possibly on a functional basis, some of the other constituents such as plasma proteins, soluble collagen, and neutral mucoproteins are more important. Another assumption which may be fallacious is that changes in serum mucoprotein (which is neutral) reflect changes in acid mucopolysaccharide of the ground substance; chemically the two are quite distinct.

Although numerous, presumedly distinct mucopolysaccharides of connective tissue have been identified, the principal ones appear to be hyaluronic acid, chondroitin sulfuric acid, and heparin. These have in common the fact that they are polymers of high molecular weight and are composed, among other

moieties, of hexosamines (glucosamine in the case of hyaluronic acid; galactosamine in the case of chondroitin sulfate) and of glucuronic acid. Chondroitin sulfuric acid comprises 12 to 14 per cent of cartilage, but with this exception the mucopolysaccharides are present only in low concentration in connective tissues. Chondroitins B and C, differing slightly from chondroitin A of cartilage, have been identified. Chondroitin sulfate B, which apparently is characterized by an absence of glucuronic acid, occurs in heart valves, tendon, aorta, and skin.[18] Metabolically mucopolysaccharides are rapidly turned over. Metachromatic staining of connective tissues, as by toluidine blue, is a function largely of mucopolysaccharides.[36,37] In the mammalian organism hyaluronidase has been identified with certainty only in testis.

TABLE I. CHARACTERISTICS OF COLLAGEN

(A partial tabulation)

CATEGORY BASED ON METHOD OF STUDY	CHARACTERISTIC FEATURES
Histologic properties[11a]	Tinctorial characteristics: Acid fuchsin—bright red staining Periodic acid and Schiff's reagent—faint red staining Silver methods—not well stained Dilute acids and alkalis—swelling
Electron microscopic features	640 Å periodicity
Chemical features	14% hydroxyproline by weight. Hydroxylysine also unique amino acid. Low content of tyrosine, methionine, and histidine. Absence of cystine and tryptophane. 1% hexosamine (associated carbohydrate)
Shrinkage characteristics	Temperature: 60°-65°C. (shrinkage to about 1/3 original length) Certain electrolyte solutions
Behavior toward enzymes	Attacked by pepsin Attacked by "collagenases" of *Clostridium histolyticum* and *Cl. welchii*[11b] Resistant to trypsin, chymotrypsin, papain, hyaluronidase
Isotope tracer studies of metabolic turnover rate	Relative metabolic inertia[3]
X-ray crystallography	Characteristic pattern(s)[1]
Immunologist	Lack of antigenicity of unaltered collagen,[14] viz., use for suture material

The importance of the ground substance is evident when one considers that it must be traversed by all materials entering and leaving the cells.[6b] The concept[7,34,42] that mucopolysaccharides function like a reactive gel of the ion-exchange resin group is an intriguing but as yet unproved concept. It is at least theoretically possible that changes in the concentration or state of polymerization might modify greatly the capacity of connective tissues to bind inorganic ions and water. Hyaluronic acid, highly hygroscopic in the purified

state, may be concerned in water-binding by tissues. Chondroitin sulfate, because of its highly charged anionic groups, may function[11] as a cation exchange resin.

Jackson[24] has presented data which he interprets as indicating an important role of mucopolysaccharide in the organization and certain properties of collagenous structures such as tendon. Trypsin will digest gelatin but not native collagen. The characteristic shrinkage temperature of native collagen is altered by treatment directed at the matrix.

FURTHER CONSIDERATIONS

In addition to these general features of connective tissue, which undoubtedly assists in the understanding of the heritable disorders to be discussed, there are some specific questions about the biology of connective tissue which come to mind with study of these diseases. For example, to mention only a few, in the Marfan syndrome: What does the suspensory ligament of the ocular lens have in common with the media of the aorta? What controls longitudinal growth of bone? In pseudoxanthoma elasticum: What is the nature of Brück's membrane of the eye and what does it have in common with the corium? What is the basic nature of orceinophilic staining; specifically, if it is dystrophic collagen which stains in this disease, what change has occurred to result in this tinctorial simulation of elastin? In the Ehlers-Danlos syndrome: What is responsible for the tensile strength of the skin and for its elasticity? What determines the elastic properties of the collagen and elastin molecules, and of fibers of these proteins? In osteogenesis imperfecta: What is the normal organization of apatite on collagen which accounts for the important structural properties of bone, and, in this disease, what change in collagen has occurred to disrupt the normal collagen-apatite relationship? In connection with the full discussion of each of the entities, what is known in answer to these questions and others will be presented. Unfortunately, much remains to be learned in all these areas.

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